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REVIEW ARTICLE

# Virulence Determinants of *Aeromonas* Species Implicated in Fish Diseases and Control of Infection: An overview

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## Abstract

Aeromonads are halophilic, non-spore forming, Gram-negative rods which are ubiquitous in aquaculture and foodstuffs. Members of genus Aeromonas are abundant water inhabitant bacteria that were recovered from lakes, rivers, swamps, chlorinated water as well as food stuff as fish, meat, seafood, vegetables, and processed foods. Aeromonas species are opportunistic pathogens that affect many aquatic animals and human. These pathogens cause septicaemia, ulcerative and haemorrhagic diseases, and mortality in different fish species. They possess large number of virulence factors in addition to inherent resistance to various antimicrobials and ability to form biofilms with the help of quorum sensing. This review focuses on the pathogenic potentials of Aeromonas species which regarded as multifactorial and dependent on the presence of different virulence factors that enable bacteria to colonize, invade, and defeat the host's immune defences. This review also provides an update on the taxonomy, ecology, and control of Aeromonas infection in fishes.

Keywords: Aeromonas spp.; Virulence factors; Probiotics;, Prebiotics; Genotyping

## INTRODUCTION

*Aeromonas* species belongs to the class Gamma-proteobacteria, order Aeromonadales and the family Aeromonadaceae [1]. These bacteria are, facultative anaerobic, motile , non-sporulating Gram negative bacilli [2]. Aeromonads are primarily aquatic organisms occurring naturally in different freshwater bodies that include rivers, water streams and lakes[3]. However, these organisms do not occur in water with a very high salinity, geothermal springs or extremely polluted rivers [4]

Members of genus *Aeromonas* are opportunistic pathogens that affect many aquatic animals [5,6]. Diseases and mortality in different fish species were attributed to *Aeromonas* species [7]. *A. caviae*, *A. hydrophila*, *A. salmonicida*, *A. sorbia*, and *A. veronii* were regarded as the most important causes of disease and mortality in fish [8–10]. Aeromonads have many virulence factors that allow bacteria to invade the host immune system and contribute to the pathogenicity of this organism. Some of these factors are serine protease (*ser*), aerolysin (*aer*), lipase (*lip*), cytotonic heat–stable enterotoxin (*ast*), hemolysin (*hly A*), cytotoxic enterotoxin (*act*) and temperature-sensitive protease (*eprCAI*). To determine the pathogenic effect of *Aeromonas* species these virulence genes have been used [11–14]. The major virulence factors correlated with *Aeromonas* species are S-layers, surface polysaccharides, iron binding machinery, extracellular enzymes and exotoxins, secretion systems and adhesins [15]. Since the occurrence of these virulence factors is strain specific, virulence potential of different *A. hydrophila* strains was characterized by the incidence of their virulence genes [16–19]. A lot of bacterial diseases appear in fish farms due to increased stocking density of fish, which led to extensive antibiotic usage for their treatment

[20, 21]. Unwise use of antibiotics led to the emergence of antibiotic resistance among pathogenic bacteria in fish farming [22,23]. *Aeromonas* species are used as a good indicator for analysing the occurrence and antimicrobial resistance of bacteria in fish farms [1, 22].

## 1. Taxonomy and Classification of *Aeromonas* species

The genus *Aeromonas*, belonging to the class Gamma- proteobacteria and the family Aeromonadaceae, contains Gram-negative, non-sporulating, facultative anaerobic bacilli [24,25]. Until 1970, according to physiological characteristics and host range; *Aeromonas* species were classified into two main groups. Motile Aeromonads is the first group with optimum growth temperature at 35–37ºC, this group is known as *A. hydrophila* and it predicted to produce human infections. The second group is non motile aeromonad which grows at 22–28ºC, it is called *A. salmonicida* and produces infections in fishes [26]. Thereafter, new species was added to the genus *Aeromonas* followed by reclassification of pre-existing taxa [27]. Earlier, *Vibrio* species, *Aeromonas* species and *Plesiomonas shigelloides* were included in the family called Vibrionaceae but the recent genetic findings have provided enough information to change this idea and placed *Aeromonas* species in family Aeromonadaceae [28]. Based on 16S ribosomal RNA similarity and DNA-DNA hybridization; members of genus *Aeromonas* were classified [29]. DNA–DNA hybridization assay were used to classify *Aeromonas* species into multiple hybridization groups within each of the mesophilic species [24]. Until March 2016, 31 *Aeromonas* species had been discovered [10]. The genus currently includes 36 species [30].

## 2. Identification of *Aeromonas* species

### *Phenotypic identification*

*Aeromonas* species are facultative anaerobes that produce characteristic colonies with hemolysis on blood agar or not. They do not require sodium ion for growth and tolerated up to 4% NaCl in the culture medium [31].

Genus Aeromonas is phenotypically identified by Gram-negative staining , oxidase positive reaction, fermentation of glucose with production of acid and gas, reduction of nitrate and growth inhibition by vibriostatic factor O/129 [32]. Because of the changing behaviour of some strains, identification to the species level using this method is complicated. Some strains recovered from diseased fish were re-identified which were identified phenotypically. The 16S rRNA PCR-restriction fragment length polymorphism (RFLP) and RNA polymerase, sigma 70 (sigma D) factor (*rpoD)* sequences were applied to reidentify the isolates and the results showed that only 35.5% were correctly identified [33]. Moreover, commercial identification kits (API 20E, Vitek, BBL Crystal Enteric/Non fermenter, and MicroScan Walk/Away systems) have frequently been utilized in laboratories, in spite of other authors decided that these kits had drawbacks [34]. Lamy et al., [35] evaluated the accuracy of six commercial kits for *Aeromonas* species identification using RNA polymerase subunit B (*rpoB)* sequencing as a reference. Molecular methods were more specific than commercial identification kits. Moreover, Soler *et al*., [36] confirmed the conclusions of the previous study that MicroScan W/A and BBL Crystal E/N systems correctly identified 14.8% and 20.3% of Aeromonas strains that were formerly identified by PCR for 16S rRNA gene -RFLP analysis, respectively.

### *Molecular identification*

#### *16S rRNA gene-based techniques*

Identification and comparison of bacterial species is now possible by the use of 16S rRNA gene which is highly conserved marker [37, 38]. Sequencing of 16S rRNA gene is the commonly applied molecular tool in clinical laboratory for genus and species identification [39]. When

trying to identify Aeromonads using this technique wariness should be taken because of the sequence variation between 16S rRNA genes in the same strain, which may reach 1.5% [40].

One of the effective tools introduced in many clinical laboratories for the identification of bacteria is Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS basically identify proteins associated with the 16S rRNA gene [41].

MALDI-TOF MS was used to identify isolates previously characterized by sequencing of *rpoB* and results showed that 100% of isolates were properly identified at genus level, and 97% at species level [42].

***Housekeeping Genes***

Proteins having crucial functions for the survival of bacteria are encoded by housekeeping genes (HKG). The ideal HKG should be present in all bacteria and should not be influenced by horizontal gene transfer [40].

The first HKG that has been used to study *Aeromonas* species was *gyrB* gene which encodes the B subunit of DNA gyrase [43]. The *rpoD* gene is a similar HKG that displays a related phylogeny to *gyrB* which encodes factor sigma S70 (that enables promoter-specific transcription initiation of RNA polymerase)[34]. Other HKGs have been described: *gyrA rpoB, dnaJ ,recA, dnaX, dnaK,cpn60, mdh, atpD, groL, gltA radA, metG, ppsA, tsF*, and *zipA* [44–50] for identification of *Aeromonas* species.

Amplified fragment length polymorphism (AFLP) analysis has repeatedly been shown to be a very useful method for classification and typing of Aeromonads [51].

Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) is one of the highly effective methods for genotyping of *Aeromonas* species as it is highly reproducible and easy to perform and does not need too expensive instruments. Therefore, it has been utilized in many epidemiological surveys [52- 54] to investigate the clonality of Aeromonas isolates.

Restriction fragment length polymorphism (RFLP) assay was used for genotyping of *A. hydrophila* using *Eco*RII and *Eco3* restriction enzymes[55].

Multilocus sequence typing (MLST) is a typing method based on the sequences analysis of 5-7 housekeeping genes to identify strains by their unique allelic profiles and so facilitates the discrimination of microbial isolates [56]. This technique is highly differentiating and reproducible when compared with other molecular tools. The generated databases can guid researchers to compare the results they obtain. The bacterial isolate genome sequence database (BIGSdb) is platform that currently manages the MLST database.

Phylogenetic grouping of *Aeromonas* species rely on the analysis of two housekeeping genes (*rpoD* and *gyrB*) were consistent with the described taxonomy of *Aeromonas* species [34].

## 3. Pathogenesis and virulence factors

During host pathogen interaction, microbes multiply, survive, and combat the host's immunity. The clinical signs that are observed from *Aeromonas* infections indicate a complicated network of mechanisms forming of a multifactorial process. This hypothesis was strengthen by several studies as the strain, infection route, and animal model affect the virulence of *Aeromonas* species [7, 14, 57]. Virulence factors of *Aeromonas* species include:

### *3.1. Surface structural components*

**Capsule**

The outer membrane of the bacterial cell is enclosed by capsule which is formed of polysaccharides and water. Polysaccharides are formed by repetitions of monosaccharides which are bind to each other by glycosidic bonds forming homo- or

hetero-polymers. The variation of capsule forming monosaccharides, probable change, and various linkage are responsible for the diversity and structural complexity [58].

The function of capsule as pathogenic factor is reducing opsonization and hence hindering phagocytosis [59].

**S-layers**

Wide range of bacteria forms S-layer which is surface protein or glycoprotein forming the outermost cell envelope. S-layers have several functions that are related to virulence. It has a major role in adhesion, and protect the bacteria from phagocytosis [60].

**Adhesins**

The ability of bacteria to stick and colonize the host mucosa is considered a crucial step in the infection. The first most important step in the process of infection is adhesion of bacteria to host cell. Bacteria attach to host cells and change their defence mechanisms by the initiation of colonization process. *Aeromonas* species has two class of adhesions which enable it to bind to receptors on the host cell surface [61].

**Filamentous Adhesins: Fimbriae/Pili**

Bacterial cell surface has filamentous structures called fimbriae or pili which consists of protein subunits called pilin. Pili have many functions rather than adhesion like cell aggregation, phage binding, biofilm formation, and transfer of DNA. [62].

**Non-filamentous Adhesins**

These are macromolecules on the surface of bacterial cell surface and act as adhesins, as lipopolysaccharide (LPS), S - layer and outer membrane proteins. The porins are example of outer membrane proteins which act as a lectin-type adhesins that help bacteria to attach to carbohydrate-rich surfaces as red blood cells and possibly human intestinal cells [63].

### *3.2. Extracellular proteins and enzymes*

The process of interaction between host cells and pathogenic *Aeromonas* species is revealed by the production of enzymes and toxins and their secretion out the cells, like, lipases, proteases, enterotoxins, hemolysins and Shiga toxins [8, 15, 64]. Wide range of exotoxins is produced by *Aeromonas* species. Not all toxins are produced by all strains. Moreover, toxin genes are expressed under specific growth conditions in some strains [15].

Cytotonic (change the target cell morphologically without killing) and cytotoxic enterotoxins have been found in *Aeromonas* species 7, 15]. In *A. hydrophila* the cytotoxic enterotoxin (act) has an important role in Aeromonas infections because it inhibits phagocytosis, triggers hemolysis, and rises the level of interleukin (IL-1β) and tumour necrosis factor α (TNF-α) [65]. Proteolytic nicking near the C-terminus activates the inactive secreted protein. The active toxin attaches to a glycoprotein on the target cell surface and accumulates pores in the host cytoplasmic membrane causing cell death [66].Two classes of hemolysins at least are produced by *Aeromonas* species; α-hemolysins and ß-hemolysins. The α-hemolysins are formed in the stationary phase of growth and are responsible for incomplete lysis of erythrocytes and reversible cytotoxic effects. ß-hemolysins are formed in the exponential phase of growth cycle. They are pore forming toxins leading to complete destruction and lysis of red blood cells [15].

Hemolysins make holes in the target cell membrane leading to their osmotic lysis [67]. The prototype hemolysin of the genus is aerolysin which is encoded by a gene called *aerA* [8]. Type two secretion system (T2SS) is responsible for formation of aerolysin which is known to be sec-dependent where aerolysin is transcribed as a pro-aerolysin that go through several processes of maturation before the exportation of the active aerolysin to the external environment [68].

Extracellular proteases enable *Aeromonas* species to survive in various habitats and facilitate ecological interactions with the host. Protease enhances the pathogenicity because they facilitate invasion either by toxin activation or direct host tissue damage [8, 15]. Three types of proteases are secreted by *Aeromonas* species: metalloprotease (ahp, aphB), acetylcholinesterase, and serine protease (aspA) [69, 70]. Furthermore, they can assist the formation of infection by disabling the initial host defences, like, inactivating the complement, or by supplying nutrients for cell reproduction [15].

Lipases are formed by various bacterial species. *Aeromonas* species produce lipases in the surrounding environment to hydrolyse membrane lipids leading to impairment of many immune system tasks through free fatty acids produced by lipolytic activity. Lipase can digest the membranes of erythrocytes and induce their lysis [9, 16].

### *3.3. Secretion Systems*

Gram-negative bacteria have inner cytoplasmic membrane and an outer membrane containing LPS with a thin peptidoglycan layer in between. The space between the two cytoplasmic membranes is called the periplasmic space. Gram-negative bacteria had different types of secretion systems: type I, II, III, IV, V, and VI to transfer proteins to the extracellular environment or to the cell surface [71].

Many Gram-negative bacteria have type III and VI secretion systems which deliver their toxic proteins (effectors) directly into the target host cells [16, 31, 72, 73].

Studies showed that infections by *A. hydrophila* and *A. salmonicida* strains having mutation in T3SS had a reduced virulence than the wild strains [74, 75].

Type four secretion system (T4SS) is the only known secretion system that can transport DNA in addition to proteins [76]. T4SS performs important role in the dissemination of virulence and resistance genes [8, 15].

T3SS, T4SS, and T6SS are able to insert effector proteins directly into the cytoplasm of the host cell, even though T6SS has been discovered in nonpathogenic and symbiotic organisms or [77]. After complete genome sequencing of *A. hydrophila*, T6SS was identified even though its role in virulence was undiscovered [69]. The role of T6SS in the virulence of *A. hydrophila* was then discovered [78]. T6SS was then shown to have antibacterial function in multiple bacterial infections for elimination of competing bacteria [79]. *Shigella sonnei* T6SS conferred privilege when competing with *S. flexneri* and *Escherichia coli* and this privilege was diminished in mutants defective in T6SS [80].

### *3.4. Metal ions*

Normal biological processes of microorganisms require the presence of metal ions. They play an essential part in the interplay between host and pathogen. In the progression of an infection, the host inhibits the availability of essential metals, by disabling the metal dependent biological processes of the M.O which compensate this inadequacy by producing alternative proteins [81, 82]. Iron gain mechanisms are recognized to play a vital role in the progression of the infection. Low level of iron makes pathogenicity of bacteria more difficult [8].

Siderophore-dependent mechanism is one of the mechanisms by which *Aeromonas* species sequestrate iron from the host tissues. A functional group having high affinity to iron ions is provided by Siderophores that require certain membrane-bound receptors and a special cell- machinery to make this essential element available and incorporate the iron ions into the microbial metabolism. Other mechanisms independent on Siderophore include bacterial membrane bound protein that binds host iron [8, 15, 83].

Mesophilic *Aeromonas* produce either enterobactin siderophores or amonabactin siderophores, but never produce both. The enterobactinis are discovered in various

Gram-negative bacteria, but the amonabactin is identified only in *Aeromonas* species [84].

## 4. Quorum sensing

Quorum sensing (QS) is a bacterial communication system for organizing genetic expression in response to cell population, this system enables bacteria to evade the host immune system [85,8]. Expression of virulence genes, antibiotic production, plasmid conjugation and biofilm formation in *Aeromonas* species, can be induced by QS system [86- 89]. Bacteria produce substances which act as a chemical signal. In Gram-negative bacteria, these chemicals are basically acylated homoserine (AHLs), AHL in *Aeromonas* can change the host immune response [90, 89, 8, 91].

Chan *et al*., [92] reported 159 sequences of QS-related genes in *A. veronii*, which increase its virulence. Nowadays, Liu *et al*., [93] demonstrated that the formation of biofilm in *A. salmonicida* is affected by the infection with the asaI-mutant (failed to produce the short chain AHLs signal). Recently, Blöcher *et al*., [94] developed anti-QS compounds to inhibit biofilm formation of the resistant *A. caviae* strain Sch3. This study helps control the bacterial antibiotic resistance problem.

## 5. Fish diseases caused by *Aeromonas* species

*Aeromonas* species have an important role in fish diseases, and this has been known for decades, mainly there are two types of *Aeromonas* species responsible for fish disease. *A. salmonicida* is considered the causative agent of furunculosis, which has been considered the most important fish diseases in aquaculture [95, 96]. It was thought in the past, the infection affected salmonids only, but by the time it is known to affect fresh and marine water fishes [97,-96]. Fish suffer from furunculosis show some symptoms like skin hyperpigmentation, lack of appetite, lethargy, presence of the typical furuncles, septicaemia, exophthalmia,petechiae, anaemia, ascites and haemorrhagic lesions in the gills, nares, fins, vent, muscles, and internal organs [97,96]. Mesophilic motile *Aeromonas* species cause another fish disease known as ‘motile Aeromonas septicaemia or epizootic ulcerative syndrome which shows similar clinical signs as furunculosis, but some time injury may be seen only in the internal organs or skin [97-,98].

## 6. Control of Aeromonas infection in fish

To control the extensive use of antibiotics in fish farms and their possible negative impacts on the environment and public health, a continuous search for other alternative strategies is required. Even when the antibiotic concentrations in fish diet are below the minimum inhibitory concentration, the prolonged existence of antibiotics in water, combined with high numbers of bacteria in the polybacterial environments as the sediment, pond, or biofilm put selective pressure on bacteria and allow the exchange of resistance genes between them [99,100]. *Aeromonas* species may continue being adhered to biofilms on abiotic or biotic surfaces, and its existence with *E. coli* in mixed biofilms encourages the exchange and distribution of antimicrobial resistance genes [101]. The alternative strategies include:

### 6.1. Probiotics

Probiotics are combination of living microorganisms (bacteria and yeasts) that, when administered in sufficient amounts, confer a health benefit on the host [102]. The beneficial activities of probiotics were attributed to modification of intestinal microbiota, production of antitoxin substances or antibacterial (bacteriocins and organic acids), immune system modulation and competition with pathogens for nutrients, and adhesion to intestinal mucosa [103]. There was an increase of the survival rate and protective effect of probiotic against Aeromonas species. The amplitude of the survival rate between the probiotic and control groups varied considerably and depended on the probiotic species, the feeding dosages, and durations [104]. Relatively high level of protection against *A. hydrophila* was recorded in Nile tilapia for each probiotic agent *Bacillus pumilus* or

mixture of *Lactobacillus acidophilus*, *Bacillus subtilis*, Saccharomyces *cerevisiae*, and *Aspergillus oryzae* at the end of the second month more than that obtained at the end of the first month of the feeding trial [105].

Furthermore, it has been shown that the combination of multispecies probiotics of *S. cerevisiae, B. subtilis,* and *Lactococcus lactis* [106] or *B. subtilis, L. plantarum,* and *Pseudomonas. aeruginosa* [107] improves health status more effectively than the incorporation of a monospecies probiotic in the diet.

**6.2. Prebiotics**

Prebiotics are indigestible fibers that are selectively used by host microflora to confer health benefits and enhance growth performance due to the by-products generated from their fermentation by gut beneficial bacteria, such as altering the composition of the microbiota, inhibiting pathogens, stimulating immune responses, and improving resistance to stress factors [108-109]. ß-glucan (ß-1,3-glucan or ß-1,6-glucan) which is extracted from the cell wall of *S. cerevisiae* was one of the most important prebiotics used to prevent disease in freshwater fish by *Aeromonas* species. when added to the basal diet [110-111]. Feeding fish with ß glucan at 1 to 2 g/ kg diet for at least 2 weeks appeared to be ideal to induce high protection and stimulate immune response in different Aeromonas infected freshwater fishes including rainbow trout, common carp, and Nile tilapia [112-113]. The immunomodulatory mechanisms of prebiotics in augmentation of fish immunity need to be further investigated. Some studies shown that a ß-glucan supplemented diet could display variable gene expression levels of some immune and inflammation-related cytokines in fish infected with *Aeromonas* species . The response depended on the organ, with downregulation in the gut and an upregulation in the spleen and kidney [112,114,115]. Despite a preventive effect against Aeromonas infection in some investigations, no significant effect of dietary ß-glucan on immune parameters (leucocyte subpopulations, lysozyme activity, alternative complement activity (ACH50)) assessed in serum of rainbow trout and Nile tilapia has been proved [112, 114,116] .

**6.3. Synbiotics**

Synbiotics are feed supplements consist of a mixture of probiotics and prebiotics that beneficially affect the health of the host. In aquaculture, synbiotics were used to improve growth performance and feed utilization as well as increasing resistance to diseases, digestibility, and modulation of the immune system [117-119].

Different formulas of synbiotics were investigated with the purpose of studying their beneficial role to protect freshwater fish against Aeromonas infections like, *L. plantarum* JCM1149 and Fructooligosaccharides (FOS) [120], *B. subtilis* and Mannan Oligosaccharides (MOS) ) [121], inactivated *E. faecalis* and MOS [122], Bacillus spp. (*B. coagulans or B. subtilis*) and Chitooligosaccharide (COS) [123].

**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**

[1] Dias, C.; Mota, V.; Martinez-Murcia, A. and Saavedra, M. J. (2012): Antimicrobial resistance patterns of *Aeromonas* spp. isolated from ornamental fish. *Journal of Aquaculture Research and Development*, 3:1-4

[2] Piotrowska, M. and Popowska, M. (2014): The prevalence of antibiotic resistance genes among *Aeromonas* species in aquatic environments. Ann. Microbiol. 64:921–934.

[3] Dar, G. H., Kamili, A. N., Chishti, M. Z., Dar, S. A., Tantry, T. A., & Ahmad, F. (2016): Characterization of *Aeromonas sobria* isolated from fish Rohu (*Labeo rohita*) collected from polluted pond. J. Bacteriol. Parasitol. 7:1-5

[4] Hazen, T.C. ; Fliermans, C.B. ; Hirsch, R.P. and Esch ,G.W. (1978):Prevalence and distribution of Aeromonas hydrophila in the United States. Appl Environ Microbiol 36:731-8.

[5] Kozińska, A. and Pekala, A. (2010): Serotyping of *Aeromonas* species isolated from polish fish farms in relation to species and virulence phenotype of the bacteria. Bull. Vet. Inst. Pulawy, 54: 315–320.

[6] Hu, M.; Wang, N.; Pan, Z. H., Lu, C. P. and Liu, Y. J. (2012): Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls, and water environment in China. Lett. Appl. Microbiol. 55: 224–233.

[7] Hossain, M. J., Sun, D., McGarey, D. J., Wrenn, S., Alexander, L. M., Martino, M. E., Xing, Y; Terhune, J.S and Liles, M. R. (2014): An Asian origin of virulent *Aeromonas hydrophila* responsible for disease epidemics in United States-Farmed Catfish. Am Soc Microbiol. 5:1-7.

[8] Beaz-Hidalgo, R. and Figueras, M. J. (2013): *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. *Journal of fish diseases*, 36: 371–388.

[9] Yu, J.; Koo, B. H.; Kim, D. H.; Kim, D. W. and Park, S. W. (2015): *Aeromonas sobria* infection in farmed mud loach (*Misgurnus mizolepis*) in Korea, a bacteriological survey. Iran. J. Vet. Res. 16: 194–201.

[10] Chenia, H. Y. (2016): Prevalence and characterization of plasmid-mediated quinolone resistance genes in *Aeromonas* spp. isolated from South African freshwater fish. Int. J. Food Microbiol. 231: 26–32.

[11] Li, J.; Ni, X. D.; Liu, Y. J. and Lu, C. P. (2011): Detection of three virulence genes alt, ahp and aerA in *Aeromonas hydrophila* and their relationship with actual virulence to zebrafish. J. Appl. Microbiol. 110: 823–830.

[12] Yi, S. W.; You, M. J.; Cho, H. S.; Lee, C. S.; Kwon, J. K. and Shin, G. W. (2013): Molecular characterization of *Aeromonas* species isolated from farmed eels (Anguilla japonica). Vet. Microbiol. 164: 195–200.

[13] Furmanek-Blaszk, B. (2014): Phenotypic and molecular characteristics of an *Aeromonas hydrophila* strain isolated from the River Nile. Microbiol. Res. 169: 547–552.

[14] Rather, M. A.; Willayat, M. M.; Wani, S. A.; Munshi, Z. H. and Hussain, S. A. (2014): A multiplex PCR for detection of enterotoxin genes in *Aeromonas* species isolated from foods of animal origin and human diarrhoeal samples. *Journal of applied microbiology*, 117: 1721–1729.

[15] Tomás, J. M. (2012): The Main Aeromonas Pathogenic Factors. Int. Sch. Res. Network. ISRN Microbiol. 1–22.

[16] Oliveira, S. T. L.; Veneroni-Gouveia, G. and Costa, M. M. (2012): Molecular characterization of virulence factors in *Aeromonas hydrophila* obtained from fish. Pesqui. Vet. Bras. 32: 701–706.

[17] Hossain, S.; De Silva, B. C. J. J.; Dahanayake, P. S. and Heo, G.-J. J. (2018): Characterization of virulence properties and multi-drug resistance profiles in motile *Aeromonas* spp. isolated from zebrafish (Danio rerio). *Letters in applied microbiology*, 67: 598–605.

[18] Ramadan, H.; Ibrahim, N.; Samir, M.; Abd El-Moaty, A. and Gad, T. (2018): *Aeromonas hydrophila* from marketed mullet (Mugil cephalus) in Egypt: PCR characterization of b-lactam resistance and virulence genes. *Journal of applied microbiology*, 124: 1629–1637.

[19] El-Bahar, H. M.; Ali, N. G.; Aboyadak, I. M.; Khalil, S. A. E. S. and Ibrahim, M. S. (2019): Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in Oreochromis niloticus. Int. Microbiol. 22: 479–490.

[20] Guz, L. and Kozińska, (2004): Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (Cyprinus carpio L.). Bull. Vet. Inst. Pulawy, 48: 391–395.

[21] Daood, N. (2012): Isolation and antibiotic susceptibility of *Aeromonas* spp. from fish farm and farmed Carp (Dam of 16 Tishreen, Lattakia). *Journal Basic Sciences*, 28:27-39

[22] Patil, H. J.; Benet-Perelberg, A.; Naor, A.; Smirnov, M.; Ofek, T.; Nasser, A.; Minz, D., and Cytryn, E. (2016): Evidence of increased antibiotic resistance in phylogenetically diverse *Aeromonas* isolates from semi-intensive fishponds treated with antibiotics. Front. Microbiol. 7: 1875.
[23] Mulyani, Y.; Aryantha, I.N.P.; Suhandono, S. and Pancoro, (2018): Intestinal Bacteria of Common Carp (Cyprinus carpio L.) as a Biological Control Agent for *Aeromonas*. J. Pure Appl. Microbiol. 12: 601–610.
[24] Janda, J. M. and Abbott, S. L. (2010) The genus Aeromonas: Taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev.23: 35–73.
[25] Buller, N. (2014): Bacteria and fungi from fish and other aquatic animals: a practical identification manual. 2 nd CABI Publishing, p 425.
[26] Saavedra, M. J.; Figueras, M. J. and Martínez-Murcia, A. J. (2006): Updated phylogeny of the genus Aeromonas. Int. J. Syst. Evol. Microbiol. 56: 2481–2487.
[27] Mailafia, S. and Ajogi, I. (2009): Biochemical characteristics and virulence factors of Nigerian strains of *Aeromonas* species isolated from cases of diarrhoea and domestic water. J. Pure Appl. Microbiol. 3: 41–44.
[28] Colwell, R. R.; MacDonell, M. T. and De Ley, J. (1986): Proposal to recognize the family Aeromonadaceae fam. nov. Int. J. Syst. Bacteriol. 36: 473–477.
[29] Martin-Carnahan, A. and Joseph, S. W. (2005): Aeromonadales ord. nov. In bergey's manual® of systematic bacteriology; Springer US, 2: 556–587.
[30] Fernández-Bravo, A. and Figueras, M. J. (2020): An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity. Microorganisms, 8 :129.
[31] Popoff, M. Genus III. (1984): *Aeromonas Kluyver* and Van Niel 1936, 398AL. In bergey's manual of systematic bacteriology. 545–548.
[32] Abbott, S. L.; Cheung, W. K. W. and Janda, J. M. (2003): The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. J. Clin. Microbiol. 41: 2348–2357.
[33] Beaz-Hidalgo; R., Alperi, A.; Buján, N.; Romalde, J. L. and Figueras, M. J. (2010): Comparison of phenotypical and genetic identification of *Aeromonas* strains isolated from diseased fish. Syst. Appl. Microbiol. 33: 149–153.
[34] Soler, L., Yanez, M. A., Chacon, M. R., Aguilera-Arreola, M. G., Catalán, V., Figueras, M. J., & Martínez-Murcia, A. J. (2004): Phylogenetic analysis of the genus *Aeromonas* B based on two housekeeping genes article in. Int. J. Syst. Evol. Microbiol. 54: 1511–1519.
[35] Lamy, B.; Laurent, F.; Verdier, I.; Decousser, J.W.; Lecaillon, E.; Marchandin, H.; Roger, F.; Tigaud, S.; de Montclos, H. and Kodjo, A. (2010): Accuracy of 6 commercial systems for identifying clinical *Aeromonas* isolates. Diagn. Microbiol. Infect. Dis. 67: 9–14.
[36] Soler, L.; Marco, F.; Vila, J.; Chacón, M. R.; Guarro, J. and Figueras, M. J. (2003): Evaluation of two miniaturized systems, microScan W/A and BBL crystal E/NF, for identification of clinical isolates of *Aeromonas* spp. J. Clin. Microbiol. 41: 5732–5734.
[37] Stackebrandt, E.; Frederiksen, W.; Garrity, G. M.; Grimont, P. A. D.; Kämpfer, P.; Maiden, M. C. J.; Nesme, X.; Rosselló-Mora, R.; Swings, J.; Trüper, H. G.; Vauterin, L.; Ward, A. C. and Whitman, W. B. (2002): Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int. J. Syst. Evol. Microbiol. 52: 1043–1047.
[38] Woo, P. C. Y.; Lau, S. K. P.; Teng, J. L. L.; Tse, H. and Yuen, K. Y. (2008): Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect. 14: 908–934.
[39] Janda, J. M. and Abbott, S. L. (2007): 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. J. Clin. Microbiol. 45: 2761–2764.

[40] Morandi, A.; Zhaxybayeva, O.; Gogarten, J. P. and Graf, J. (2005): Evolutionary and diagnostic implications of intragenomic heterogeneity in the 16S rRNA gene in *Aeromonas* strains. J. Bacteriol. 187: 6561–6564.

[41] Pérez-Sancho, M.; Cerdá, I., Fernández-Bravo, A.; Domínguez, L., Figueras, M. J.; Fernández-Garayzábal, J. F. and Vela, A. I. (2018): Limited performance of MALDI-TOF for identification of fish *Aeromonas* isolates at species level. J. Fish Dis. 41: 1485–1493.

[42] Chen, P. L.; Wu, C. J.; Chen, C. S.; Tsai, P.J.; Tang, H. J. and Ko, W. C. (2014): A comparative study of clinical *Aeromonas dhakensis* and *Aeromonas hydrophila* isolates in southern Taiwan: *A. dhakensis* is more predominant and virulent. Clin. Microbiol. Infect. 20: O428- O434.

[43] Yanez, M. A., Catalán, V., Apráiz, D., Figueras, M. J., and Martínez-Murcia, A. J. (2003): Phylogenetic analysis of members of the genus Aeromonas based on gyrB gene sequences. Int. J. Syst. Evol. Microbiol. 53: 875–883.

[44] Küpfer, M., Kuhnert, P., Korczak, B. M., Peduzzi, R., & Demarta, A. (2007): Genetic relationships of *Aeromonas* strains inferred from 16S rRNA, gyrB and rpoB gene sequences. Artic. Int. J. Syst. Evol. Microbiol. 56: 2743–2751.

[45] Sepe, A.; Barbieri, P.; Peduzzi, R. and Demarta, A. (2008): Evaluation of *recA* sequencing for the classification of *Aeromonas* strains at the genotype level. Lett Appl Microbiol. 46: 439–444.

[46] Miñana-Galbis, D.; Urbizu-Serrano, A.; Farfán, M.; Fusté, M. C. and Lorén, J. G. (2009): Phylogenetic analysis and identification of *Aeromonas* species based on sequencing of the cpn60 universal target. Int. J. Syst. Evol. Microbiol. 59: 1976–1983.

[47] Minana-Galbis, D., Farfan, M., Lorén, J. G., & Fuste, M. C. (2010). Proposal to assign Aeromonas diversa sp. nov. as a novel species designation for Aeromonas group 501. *Systematic and applied microbiology*, *33*(1), 15-19.

[48] Martinez-Murcia, A.J.; Monera, A.; Saavedra, M.J.; Oncina, R.; Lopez-Alvarez, M.; Lara, E. and Figueras, M.J. (2011): Multilocus phylogenetic analysis of the genus *Aeromonas*. Syst. Appl. Microbiol. 34: 189–199.

[49] Martino, M.E.; Fasolato, L.; Montemurro, F.; Rosteghin, M.; Manfrin, A.; Patarnello, T.; Novelli, E. and Cardazzo, B. (2011): Determination of microbial diversity of *Aeromonas* strains on the basis of multilocus sequence typing, phenotype, and presence of putative virulence genes. Appl. Environ. Microbiol. 77: 4986–5000.

[50] Roger, F.; Marchandin, H.; Jumas-Bilak, E.; Kodjo, A. and Lamy, B. (2012): Multilocus genetics to reconstruct aeromonad evolution. BMC Microbiol. 12 :1-23

[51] Huys, G.; Kämpfer, P. and Swings, J. (2001): New DNA-DNA hybridization and phenotypic data on the species *Aeromonas ichthiosmia* and *Aeromonas allosaccharophila*: *A. Ichthiosmia* Schubert et al. 1990 is a later synonym of *A. Veronii* Hickman-Brenner et al. 1987. Syst. Appl. Microbiol. 24: 177–182.

[52] Khor, W.C.; Puah, S.M.; Koh, T.H.; Tan, J.A.M.A.; Puthucheary, S.D. and Chua, K.H. (2018): Comparison of clinical isolates of *Aeromonas* from Singapore and Malaysia with regard to molecular identification, virulence, and antimicrobial profiles. Microb. Drug Resist. 24: 469–478.

[53] Szczuka, E. and Kaznowski, A. (2004): Typing of clinical and environmental *Aeromonas* spp. Strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus sequence PCR. J. Clin. Microbiol. 42: 220–228.

[54] Tartor, Y. H.; EL-Naenaeey, E.-S.Y.; Abdallah, H.M.; Samir, M.; Yassen, M.M.; Abdelwahab, A.M. (2021): Virulotyping and genetic diversity of *Aeromonas hydrophila* isolated from Nile tilapia (*Oreochromis niloticus*) in aquaculture farms in Egypt. Aquaculture, 541: 736781.

[55] Algammal, A. M.; Mohamed, M. F.; Tawfiek, B. A.; Hozzein, W. N.; El Kazzaz, W. M. and Mabrok, M. (2020): Molecular typing, antibiogram and PCR-RFLP based

detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. Pathogens, 9: 238.
[56] Jolley, K. A. and Maiden, M. C. J. (2010): Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics, 11:1-11
[57] Yu, H.B.; Zhang, Y.L.; Lau, Y.L.; Yao, F.; Vilches, S.; Merino, S.; Tomas, J.M.; Howard, S.P. and Leung, K.Y. (2005): Identification and characterization of putative virulence genes and gene clusters in *Aeromonas hydrophila* PPD134/91. Appl. Environ. Microbiol. 71: 4469–4477.
[58] Roberts, I. S. (1996): The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. 50: 285–315.
[59] Garduno, R. A.; Thornton, J. C. and Kay, W. W. (1993): *Aeromonas salmonicida* grown in vivo. Infect. Immun. 61: 3854–3862.
[60] Sleytr, U. B.; Egelseer, E. M.; Ilk, N., Pum, D. and Schuster, B. (2007): S-Layers as a basic building block in a molecular construction kit. FEBS J. 274: 323–334.
[61] Burke,V. ; Cooper, M.; Robinson, J.; Gracey, M.; Lesmana, M., Echeverria, P.and Jandas, J.M. (1984): Hemagglutination patterns of *Aeromonas* spp. in relation to biotype and source. J. Clin. Microbiol. 19: 39–43.
[62] Proft, T. and Baker, E. N. (2009): Review pili in Gram-negative and Gram-positive bacteria-structure, assembly and their role in disease. Cell Mol Life Sci., 66: 613–635.
[63] Khushiramani, R.M.; Maiti, B.; Shekar, M.; Girisha, S.K.; Akash, N.; Deepanjali, A.; Karunasagar, I. and Karunasagar, I. (2012): Recombinant *Aeromonas hydrophila* outer membrane protein 48 (Omp48) induces a protective immune response against *Aeromonas hydrophila* and *Edwardsiella tarda*. Res. Microbiol. 163: 286–291.
[64] Palma-Martínez, I.; Guerrero-Mandujano, A.; Ruiz-Ruiz, M.J.; Hernández-Cortez, C.; Molina-López, J.; Bocanegra-García, V. and Castro-Escarpulli, G. (2016): Active shiga-like toxin produced by some *Aeromonas* spp., isolated in Mexico City. Front. Microbiol. 7: 1522.[65] Chopra, A.K.; Xu, X.J.; Ribardo, D.; Gonzalez, M.; Kuhl, K.; Peterson, J.W. and Houston, C.W. (2000): The cytotoxic enterotoxin of *Aeromonas hydrophila* induces proinflammatory cytokine production and activates arachidonic acid metabolism in macrophages. Infect. Immun. 68: 2808–2818.
[66] Ferguson, M.R.; Xu, X.J.; Houston, C.W.; Peterson, J.W.; Coppenhaver, D.H.; Popov, V.L. and Chopra, A.K. (1997): Hyperproduction, purification, and mechanism of action of the cytotoxic enterotoxin produced by *Aeromonas hydrophila*. Infect. Immun. 65: 4299–4308.
[67] Galindo, C. L.; Gutierrez, C. and Chopra, A. K. (2006): Potential involvement of galectin-3 and SNAP23 in *Aeromonas hydrophila* cytotoxic enterotoxin-induced host cell apoptosis. Microb. Pathog. 40: 56–68.
[68] Latif-Eugenín, F. L. (2015): Aeromonas, un microorganismo ambiental de importancia en salud humana y animal, Universitat Rovira i Virgili, Thesis Doctoral.1-406.
[69] Seshadri, R.; Joseph, S.W.; Chopra, A.K.; Sha, J.; Shaw, J.; Graf, J.; Haft, D.; Wu, M.; Ren, Q.; Rosovitz, M.J.; Madupu, R.; Tallon, L.; Kim, M.; Jin, S.; Vuong, H.; Stine, O. C.; Ali, A.; Horneman, A. J. and Heidelberg, J. F. (2006): Genome sequence of *Aeromonas hydrophila* ATCC 7966T: Jack of all trades. J. Bacteriol. 188: 8272–8282.
[70] Reith, M.E.; Singh, R.K.; Curtis, B.; Boyd, J.M.; Bouevitch, A.; Kimball, J.; Munholland, J.; Murphy, C.; Sarty, D.; Williams, J.; Nash, J. H.; Johnson, S.C. and Brown, L. L. (2008): The genome of *Aeromonas salmonicida* subsp. salmonicida A449: Insights into the evolution of a fish pathogen. BMC Genomics, 9:1-15
[71] Henderson, I. R.; Navarro-Garcia, F.; Desvaux, M.; Fernandez, R. C. and Ala'Aldeen, D. (2004): Type V protein secretion pathway: the autotransporter story. Microbiol. Mol. Biol. Rev. 68: 692–744.
[72] Hueck, C. J. (1998): Type III protein secretion systems in bacterial pathogens of

animals and plants. Microbiol. Mol. Biol. Rev. 62: 379–433.
[73] Pukatzki, S.; Ma, A. T.; Revel, A. T.; Sturtevant, D. and Mekalanos, J. J. (2007): Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. Proc. Nat. Acad Sciences, 39: 15508-15513.
[74] Vilches, S.; Jiménez, N.; Merino, S. and Tomás, J. M. (2012): The Aeromonas dsbA mutation decreased their virulence by triggering type III secretion system but not flagella production. Microb. Pathog. 52: 130–139.
[75] Romero, A.; Saraceni, P.R.; Merino, S.; Figueras, A.; Tomás, J.M. and Novoa, B. (2016): The animal model determines the results of Aeromonas virulence factors. Front. Microbiol. 7:1574.
[76] Tseng, T. T.; Tyler, B. M. and Setubal, J. C. (2009): Protein secretion systems in bacterial-host associations, and their description in the Gene Ontology. BMC Microbiol. 9:1-9.
[77] Bingle, L. E.; Bailey, C. M. and Pallen, M. J. (2008): Type VI secretion: a beginner's guide. Curr. Opin. Microbiol.11: 3–8.
[78] Suarez, G; Sierra, J.C.; Sha, J.; Wang, S.; Erova, T.E.; Fadl, A.A.; Foltz, S.M.; Horneman, A.J. and Chopra, A.K. (2008): Molecular characterization of a functional type VI secretion system from a clinical isolate of *Aeromonas hydrophila*. Microb Pathog. 44: 344–361.
[79] English, G.; Trunk, K.; Rao, V.A.; Srikannathasan, V.; Hunter, W.N. and Coulthurst, S.J. (2012): New secreted toxins and immunity proteins encoded within the Type VI secretion system gene cluster of *Serratia marcescens*. Mol Microbiol. 86: 921–936.
[80] Anderson, M. C.; Vonaesch, P.; Saffarian, A.; Marteyn, B. S. and Sansonetti, P. J. (2017): *Shigella sonnei* encodes a functional T6SS used for interbacterial competition and niche occupancy. Cell Host Microbe, 21: 769-776.e3.
[81] Capdevila, D. A.; Wang, J. and Giedroc, D. P. (2016): Bacterial strategies to maintain zinc metallostasis at the host-pathogen interface. J. Biol. Chem. 291: 20858–20868.
[82] Palmer, L. D. and Skaar, E. P. (2016): Transition metals and virulence in bacteria. Annu. Rev. Genet. 50: 67–91.
[83] Beyers, B. R.; Massad, G.; Barghouthi, S. and Arceneaux, J. E. L. (1991): Iron acquisition and virulence in the motile aeromonads: Siderophore-dependent and -independent systems. in Experientia. 47: 416–418.
[84] Hoffmann, D.; Hevel, J. M.; Moore, R. E. and Moore, B. S. (2003): Sequence analysis and biochemical characterization of the nostopeptolide A biosynthetic gene cluster from Nostoc sp. GSV224. Gene, 311: 171–180.
[85] Schwenteit J.; Gram L.; Nielsen K.F.; Fridjonsson O.H.; Bornscheuer U.T.; Givskov M. and Gudmundsdo´ttir B.K. (2011): Quorum sensing in *Aeromonas salmonicida* subsp. A *chromogenes* and the effect of the auto inducer synthetase AsaI on bacterial virulence. Vet Microbiol. 147:389 – 397.
[86] Khajanchi B.K.; Sha J.; Kozlova E.V.; Erova T.E.; Sua´rez G.; Sierra J.C. Popov V.L.; Horneman A.J. and Chopra A.K. (2009): N-acylhomoserine lactones involved in quorum sensing control the type VI secretion system, biofilm formation, protease production, and in vivo virulence in a clinical isolate of *Aeromonas hydrophila*. Microbiol. 155: 3518–3531.
[87] Vilches S.; Jimenez N.; Toma´s J.M. and Merino S. (2009): *Aeromonas hydrophila* AH-3 type III secretion system expression and regulatory network. Appl. Environ. Microbiol. 75: 6382–6392.
[88] Kozlova E.V.; Khajanchi B.K.; Sha J. and Chopra A.K. (2011): Quorum sensing and c-di-GMP-dependent alterations in gene transcripts and virulence-associated phenotypes in a clinical isolate of *Aeromonas hydrophila*. Microbial Pathogenesis, 50: 213–223
[89] Cao Y.; He S.; Zhou Z.; Zhang M.; Mao W.; Zhang H. and Yao B. (2012): Orally administered thermostable N-acyl homoserine lactonase from Bacillus sp. strain

AI96 attenuates *Aeromonas hydrophila* infection in zebrafish. Appl. Environ. Microbiol.78: 1899–1908.
[90] Khajanchi, B.K.; Kirtley, M.L.; Brackman, S.M. and Chopra, A.K. (2011): Immunomodulatory and protective roles of quorum-sensing signaling molecules N-Acyl homoserine lactones during infection of mice with *Aeromonas hydrophila*. Infect. Immun. 79: 2646–2657.
[91] Grim, C.J.; Kozlova, E.V.; Ponnusamy, D.; Fitts, E.C.; Sha, J.; Kirtley, M.L.; Van Lier, C.J.; Tiner, B.L.; Erova, T.E.and Joseph, S.J.; et al. (2014): Functional genomic characterization of virulence factors from necrotizing fasciitis-causing strains of *Aeromonas hydrophila*. Appl. Environ. Microbiol. 80: 4162–4183.
[92] Chan, X.-Y.; How, K.-Y.; Yin, W.-F.; Chan, K.-G. (2016): N-Acyl homoserine lactone-mediated quorum sensing in *Aeromonas veronii* biovar sobria strain 159: Identification of LuxRI homologs. Front. Microbiol. 6: 5772.
[93] Liu, L.; Yan, Y.; Feng, L.; Zhu, J. (2018): Quorum sensing asaI mutants affect spoilage phenotypes, motility, and biofilm formation in a marine fish isolate of *Aeromonas salmonicida*. Food Microbiol. 76: 40–51.
[94] Blöcher, R.; Ramírez, A.R.; Castro-Escarpulli, G.; Curiel-Quesada, E.and Reyes-Arellano, (2018): A. design, synthesis, and evaluation of Alkyl-Quinoxalin-2(1H)-one derivatives as anti-quorum sensing molecules, inhibiting biofilm formation in *Aeromonas caviae* Sch3. Molecules, 23, 3075.
[95] Noga E.J. (2010): Fish diseases: diagnosis and treatment, 2nd ed. Willey-Blackwell, Singapore. 536 pages
[96] Hidalgo, R. B., & Figueras, M. J. (2012). Molecular detection and characterization of furunculosis and other Aeromonas fish infections. *Health and environment in aquaculture*, 97-132. [97] Austin, B. and Austin, D.A. (2007): Bacterial fish pathogens, disease of farmed and wild fish, 4th edn. Springer-Praxis, Chichester.
[98] Kozin´ska, A. and Pe¸kala A. (2012): Characteristics of disease spectrum in relation to species, serogroups, and adhesion ability of motile aeromonads in fish. Sci. World J., 949358.
[99] Baquero, F.; Martínez, JL. and Cantón, R. (2008): Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19:260–265.
[100] Watts,J.E.M. ; Schreier, H.J. ; Lanska, L. and Hale, M.S. (2017): The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Marine Drugs 15:1–16.
[101] Talagrand-Reboul, E.; Jumas-Bilak, E. and Lamy. B. (2017): The social life of Aeromonas through biofilm and quorum sensing systems. Front. Microbiol. 8:37
[102] Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J, and Pot, B. (2014) :Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514.
[103] Myers, D. (2007): Probiotics. J Exot Pet Med 16:195–197
[104] Bandyopadhyay, P. and Das Mohapatra, P.K. (2009): Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: on growth, nutritional quality and immunity of Catla catla (ham.). Fish Physiol Biochem 35(3):467–478.
[105] Aly, S.M. ; Mohamed, M.F. and John, G. (2008) :Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). Aquac Res 39:647–656.
[106] Mohapatra, S. ; Chakraborty, T. ; Prusty ,A.K. ; Prasad, K.P. and Mohanta, K.N. (2014): Dietary multispecies probiotic supplementation enhances the immunohematological responses and reduces mortality by *Aeromonas hydrophila* in *Labeo rohita* fingerlings. J World Aquacult Soc 45:532–544.

[107] Musthafa, M.S. ; Ali, A. R.J. ; Mohamed ,M.J. ; Jaleel, M.M.A. ; Kumar , M.S.A. and Rani ,K.U. (2016) :Protective efficacy of Azomite enriched diet in *Oreochromis mossambicus* against *Aeromonas hydrophila*. Aquaculture 451:310– 315.

[108] Ringø , E. ; Olsen, R.E. ; Gifstad , T. ; Dalmo, R.A. ; Amlund, H. ; Hemre, G. and Bakke, A.M. (2010) :Prebiotics in aquaculture: a review. Aquac Nutr 16:117–136.

[109] Patel. S. and Goyal, A. (2012): The current trends and future perspectives of prebiotics research: a review. Biotech 2: 115-125

[110] Zheng, Z.L; Wang, K.; Delbert, M.G. and Ye, J.M. (2011): Evaluation of the ability of GroBiotic®-A to enhance growth, muscle composition, immune responses, and resistance against *Aeromonas hydrophila* in Nile tilapia, *Oreochromis niloticus*. J World Aquacult Soc 42:549–557.

[111] Anjugam ,M. ; Vaseeharan, B. ; Iswarya ,A. ; Gobi, N. ; Divya, M. and Thangara, M.P. (2018) :Effect of ß-1, 3 glucan binding protein based zinc oxide nanoparticles supplemented diet on immune response and disease resistance in *Oreochromis mossambicus* against *Aeromonas hydrophila.* Fish Shellfish Immunol 76:247–259.

[112] Falco , A. ; Frost, P. ; Miest, J. ; Pionnier, N. ; Irnazarow, I. and Hoole, D. (2012) :Reduced inflammatory response to *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio L*.) fed with ß-glucan supplements. Fish Shellfish Immunol 32:1051–1057.

[113] Barros, M.M. ; Falcon, D.R. ; Orsi Rde, O. ; Pezzato, L.E. ; Fernandes, A.C. and Guimarães, I.G. (2014) Non-specific immune parameters and physiological response of Nile tilapia fed ß-glucan and vitamin C for different periods and submitted to stress and bacterial challenge. Fish Shellfish Immunol 39:188–195.

[114] Ji, L. ; Sun,G. ; Li,J. ;Wang,Y. ;Du,Y. ; Li,X. and Liu,Y.(2017) :Effect of dietary ß-glucan on growth, survival and regulation of immune processes in rainbow trout(*Oncorhynchus mykiss*) infected by *Aeromonas salmonicida*. Fish Shellfish Immunol 64:56–67.

[115] Ahmadi, P. Y., Farahmand, H., Miandare, H. K., Mirvaghefi, A., & Hoseinifar, S. H. (2014) :The effects of dietary Immunogen® on innate immune response, immune related genes expression and disease resistance of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 37:209–214.

[116] Douxfils ,J. ; Fierro-Castro, C. ;Mandiki, S.N.M. ; Emile, W. ; Tort, L. and Kestemont, P. (2017) :Dietary ß-glucans differentially modulate immune and stress-related gene expression in lymphoid organs from healthy and *Aeromonas hydrophila*-infected rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 63:285–296

[117] Cerezuela, R. ; Meseguer, J. and Esteban, M. (2011) :Current knowledge in synbiotic use for fish aquaculture: a review. J Aquac Res Dev 1:1–7

[118] Ringø , E. and Song, S.K. (2016) :Application of dietary supplements (synbiotics and probiotics in combination with plant products and B-glucans) in aquaculture. Aquac Nutr 22:4–24.

[119] Das, S. ; Mondal, K. and Haque, S. (2017) :A review on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture. J Entomol Zool Stud 5:422–429

[120] Liu, W. ; Wang, W. ; Ran, C. ; He, S. ; Yang, Y. and Zhou, Z. (2017): Effects of dietary scFOS and lactobacilli on survival, growth, and disease resistance of hybrid tilapia. Aquaculture 470:50–55.

[121] Kumar, P. ; Jain, K. and Sardar, P. (2018) : Effects of dietary synbiotic on innate immunity, antioxidant activity and disease resistance of *Cirrhinus mrigala* juveniles. Fish Shellfish Immunol 80:124–132.

[122] Rodriguez-Estrada, U. ; Satoh, S. ; Haga, Y. ; Fushimi, H. and Sweetman, J. (2013) :Effects of inactivated *Enterococcus faecalis* and mannan oligosaccharides and their combination on growth, immunity, and disease protection in rainbow trout. N Am J Aquac 75:416–428

[123] Devi, G. ; Harikrishnan, R. ; Paray, B.A. ; Al-Sadoon, M.K. ; Hoseinifar, S.H. and Balasundaram, C. (2019) :Effect of symbiotic supplemented diet on innate-adaptive immune response, cytokine gene regulation and antioxidant property in *Labeo rohita* against *Aeromonas hydrophila*. Fish Shellfish Immunol 89:687–700.

**الملخص العربى**

**محددات الضراوة لأنواع الأيروموناس المتورطة في أمراض الأسماك والتحكم في العدوي: نظره عامه**

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الأيروموناد هي عصيات، غير بوغية، سالبة الجرام وتنتشر على نطاق واسع في البيئة المائية والمواد الغذائيةأنواع . الأيروموناس منتشرة في كل مكان تنتقل عن طريق الماء. يتم عزلها من الأنهار والبحيرات والمستنقعات ومياه المعقم بالكلور وأنواع مختلفة من الطعام، مثل اللحوم والأسماك والمأكولات البحرية والخضروات والأطعمة المصنعة. تعتبر أنواع الأيروموناس من مسببات الأمراض الانتهازية التي تصيب العديد من الحيوانات المائية والبشر. هذه العوامل الممرضة هي المسؤولة عن الأمراض التقرحيه وتسمم الدم والوفيات في الأسماك المختلفة. لديهم عدد كبير من عوامل الضراوة بالإضافة إلى المقاومة المتأصلة للمضادات الحيوية المختلفة والقدرة على تكوين الأغشية الحيوية بمساعدة استشعار النصاب. تركز هذه المراجعة على الإمكانات المسببة للأمراض اللإيروموناس والتي تعتبر متعددة العوامل وتعتمد على وجود العديد من عوامل الضراوة التي تسمح للبكتيريا باستعمار وغزو وهزيمة جهاز المناعة. تهدف هذه المراجعة أيضًا إلى تحديث للمعرفة المكتسبة مؤخرًا حول التصنيف والبيئة والتحكم في عدوي الايروموناس في الاسماك.